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研究課題名（和文）Mechanism of targeted DNA cleavage and recombination by AID

研究課題名（英文）Mechanism of targeted DNA cleavage and recombination by AID

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研究成果の概要（和文）：ＡＩＤはそのＮ末端およびＣ末端を介して単量体および二量体を形成し、そしてそのＤＮＡ切断および組換え活性を調節する別個の補因子と相互作用する。 HIGMII患者に見られるC末端AID変異体は二量体化欠陥であった。 それはAIDとCSRの共因子との関連性を減少させた。 追加のＣＳＲ調節クロマチンタンパク質もまた、ｉＣｈＩＰおよび候補遺伝子ノックダウンによって単離された。 DNA切断はSMARCA4-SSRP1によって促進されたが、Brd2、SAMHD1、およびPhf5aは組換えを促進した。 ＳＡＭＨＤ１調節ｄＮＴＰバランスは、ＤＮＡ修復およびゲノム安定性にとって重要であるように思われた。

研究成果の学術的意義や社会的意義
A novel competitive regulatory mechanism of AID has been observed, that help explain CSR impairment in HIGMII patient. Since distinct chromatin proteins are involved in AID's DNA-break and repair regulation, they are valuable future drug target to modulate genomic recombination.

研究成果の概要（英文）：Antibody gene diversification by SHM and CSR requires AID induced DNA break and recombination at the IgH locus. How AID exerts such functions, and the genomic stability is regulated are poorly understood.

AID's N- and C-terminus played important role in monomer and dimer formation. They interacted with specific co-factors, which differentially modulated DNA break and recombination. The C-terminal AID mutant, found in HIGMII patient, was dimerization defective and reduced association of AID with CSR co-factors. Novel CSR regulatory chromatin proteins were also identified by iChIP and candidate gene knockdown. While AID-induced DNA break was facilitated by SMARCA4-SSRP1 chromatin complex, Brd2, SAMHD1, Phf5a promoted recombination. Interestingly, the dNTPase activity of SAMHD1 was found to be critical to promote DNA repair during CSR and IgH/cMyc translocation.

研究分野：Molecular Immunology

キーワード：AID　CSR　Recombination　hnRNPK　hnRNPL　Phf5a　Samhd1　Brd2

１．研究開始当初の背景

Antigen activated mature B cells undergo antibody gene diversification through a complex DNA break and recombination mechanism induced by Activation-Induced Cytidine Deaminase (AID) [1,2]. While the N-terminal of AID is responsible for DNA break required for Somatic Hyper Mutation (SHM) and Class Switch Recombination (CSR), the C-terminal of AID is essential for the recombination phase of CSR [3,2]. Although a number of interacting proteins were identified [2,4], the domain specific function of AID remains elusive.

Our earlier study indicated that histone chaperones and histone posttranslational modifications play important role in CSR at the context of target genomic locus [5-8]. However, the information was not sufficient enough to explain highly locus specific programmed genomic rearrangement. Since many oncogenic mutations and translocations in B cells are originated during CSR [9-11], the mechanistic understanding on AID's action, and the local chromatin factors that influence the recombination efficiency are of great importance.

２．研究の目的

How AID exerts two distinct functions, DNA break and recombination through its N- and C-terminus, respectively is unknown. To address this question it is necessary to analyze AID's structure-function and its relation with the function specific various RBP co-factors.

How AID induced programmed DNA break-repair occurs in a specific target locus is also unknown. To understand the nature of break-point recombination complex, it is important to identify those chromatin factors regulate either DNA break and/or repair. Therefore, the aim was to identify those regulatory proteins.

３．研究の方法

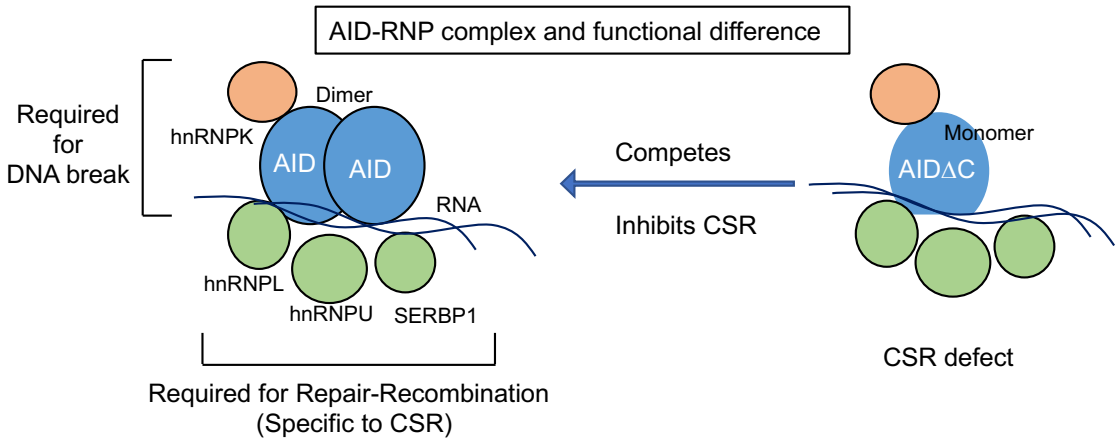
Direct visualization of AID-AID interaction or dimerization in living cells was examined by Bimolecular fluorescence complementation (BiFC) assay [12,13]. The dimer formation and the interaction of AID with the RNA binding proteins were validated by conventional immunoprecipitation (IP). Various mutants were generated by QuickChange mutagenesis and fused to Flag or Myc epitope as necessary. A knock-in B cell line expressing hyper IgM syndrome type 2 AID mutants was generated by CRISPR/Cas9 mediating engineering of the AID locus. In order to isolate CSR specific protein complex, iChIP strategy [14] was employed to pull down proteins from the acceptor Ig-switch region from B cell. Gene knockdown, ChIP, DNA break/repair assays are done based on established laboratory protocols [5-8].

４．研究成果

(1) To examine AID-AID interaction, BiFC assay was first established using APOBEC proteins that are structurally known to form dimer or tetramer, followed by analysis of wild type and AID mutants. The CSR defective AID, truncated or mutated at the C-terminus, was unable to form

dimer by the assay, which was reconfirmed by conventional IP. The N- and C-termini of AID were also found to be important to form RNP complex formation with the co-factors involved either in DNA break (hnRNPK) or repair/recombination (hnRNPL, hnRNPU, Serbp1).

In HIGMII patient, AID was found to be C-terminally mutated in one of the alleles. Despite presence of an intact AID allele, CSR was not observed, and the phenomenon was known as the dominant negative effect of the C-terminal mutant. Expressing wild type and mutant AID simultaneously in AIDKO splenic B cells, it has been confirmed that the CSR by wild type AID can be inhibited by the expression of the C-terminal mutant. To reproduce further the HIGMII condition, a C-terminal mutant was expressed from one of the AID alleles in a B cell line. Co-IP analysis showed that, the C-terminal mutant competes with the wild type to bind recombination but not the DNA break specific co-factors. Based on this study a working model has been proposed to explain the dominant negative effect and the function of AID C-terminus in CSR.

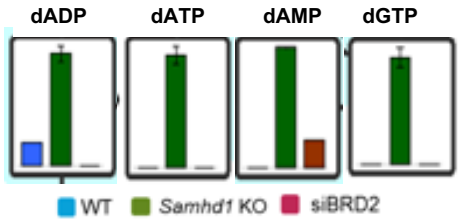


(2) We hypothesized that the CSR complex at the IgH locus is constituted by several chromatin reader proteins that locally constitute a unique recombination complex. To identify such chromatin factors, two approaches were adopted; (a) Candidate gene knock down, and (b) Locus specific protein complex isolation. Screening of Bromo and PHD domain proteins identified, Brd2 and Phf5a, respectively. On the other hand proteomics analysis of the switch α region identified several proteins of known and unknown functions in CSR. Interestingly, the cellular dNTP pool regulator SAMHD1 was found in the protein-complex. The dNTPase activity of SAMHD1 was critically required for both CSR and IgH/cMyc chromosomal translocation, suggesting that the lower dNTP level is favorable for recombination. Further study is required to fully understand the recombination complex, and the role of individual protein in the regulation of AID induced genomic instability and antibody gene diversification.

Identification of 3 chromatin factors for CSR

Gene KD	CSR	IgH/ cMyc	DNA break	DNA repair
1. Brd2	⇩	⇩	◯	⇩
2. Phf5a	⇩	⇩	◯	⇩
3. Samhd1	⇩	⇩	◯	⇩

Samhd1 loss in B cell elevates purine dNTP pool. Altered DNA repair blocks CSR and IgH/cMyc translocation induced by AID



Concluding remark

Current study suggests that the specific action of the AID is likely regulated by distinct RNAP complexes. The C-terminal of AID is not only responsible for dimer formation; it also supports CSR specific RNP complex formation. On the other hand site-specific recombination complex hold several chromatin proteins, which individually or coordinately impact AID induced genomic instability.

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５．主な発表論文等

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〔図書〕（計　0　件）

〔産業財産権〕 n/a

〔その他〕ホームページ等

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６．研究組織

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